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Toxin-producing Microcystis aeruginosa: A trade-off in the vertical distribution of three Daphnia species as predicted by the ideal free distribution with costs model

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TOXIN-PRODUCING *MICROCYSTIS AERUGINOSA*: A TRADE-OFF IN THE VERTICAL DISTRIBUTION OF THREE *DAPHNIA* SPECIES AS PREDICTED BY THE IDEAL FREE DISTRIBUTION WITH COSTS MODEL

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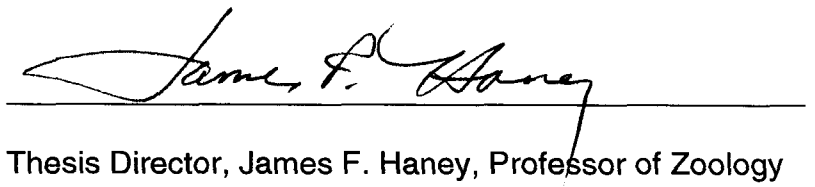

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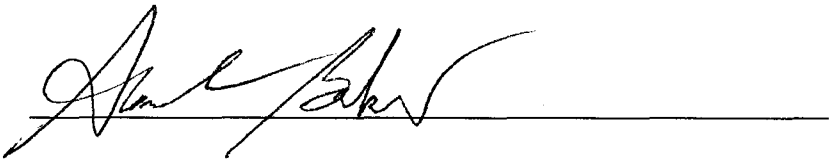

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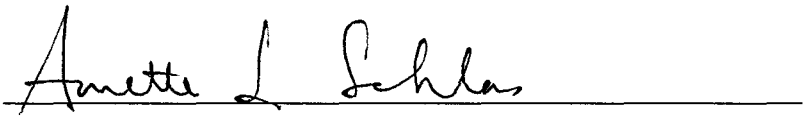

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ABSTRACT

Toxin-Producing *Microcystis aeruginosa*: A trade-off in the vertical distribution of three *Daphnia* species as predicted by the Ideal Free Distribution with Costs Model

by

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University of New Hampshire, December 2006

Daphnia hyalina, *D. galeata*, and *D. ambigua* were exposed to the cyanobacterium *Microcystis aeruginosa* in 30 cm temperature-stratified columns. Changes in egg ratio, lipid index, length, and growth were recorded over 3 day experiments. Spatial distributions were recorded during 6-hour constant light experiments and 3 day fluctuating light experiments. *D. hyalina* and *D. galeata* stayed in the upper half of the column in temperatures of 18-22°C when exposed to *M. aeruginosa*. *D. ambigua* remained between 10-19°C regardless of food type. For across all experiments, depth and temperature ranges were narrowest in treatments with *M. aeruginosa*. *D. ambigua* incurred costs to both spatial distributions and fitness parameters. *D. hyalina* and *D. galeata* incurred costs to fitness but not spatial distribution when *M. aeruginosa* was present in the experimental system. Future work should investigate the natural lake systems containing the *Daphnia* used in these experiments to tease out the influences on temperature and depth choices of the animals.

CHAPTER 1

INTRODUCTION

Filter feeding *Daphnia* (subphylum: Crustacea, suborder: Cladocera) are a key component of lake food webs functioning as grazers, competitors, and prey. *Daphnia* graze on phytoplankton (Sarnelle 1992) which is often most abundant in the warm, euphotic epilimnetic layer of a lake (Gessner 1948; Lampert et al. 2003; Reichwaldt and Stibor 2005). *Daphnia* grazing is a key element for controlling phytoplankton populations (Dodson and Hanazato 1995) as seen in Lake Washington, Seattle, WA, during the summer of 1976 when increased grazing by *Daphnia* (Edmondson and Litt 1982) doubled the water transparency. Lake Brunnsee, Germany, an increase in the epilimnetic phytoplankton population was observed in response to moving *D. hyalina* to the hypolimnion (Reichwaldt and Stibor 2005). As competitors, large-sized *Daphnia* species can diminished rotifer densities through both competitive exploitation and mechanical interference (Gilbert 1988); however, such interactions do not appear to confer a competitive advantage to the *Daphnia*. In many lakes where fish are present, daphnids compose a large part of fish diets (Lampert 1993; Mills et al. 1986). As *Daphnia* are important lake herbivores, competitors, and prey, an understanding of their habitat requirements is crucial.

Daphnia living in the warm epilimnetic layer of a lake have enhanced reproduction and growth rates. In previous research, *Daphnia pulex* (McClendon

1910) and *D. galeata mendotae* (Hall 1964) kept in water warmer than 25°C in a controlled culture reached reproductive maturity in as few as 6 days. However, more recent studies have suggested that while reproduction might occur earlier at higher temperatures, increased population growth is reached when daphnids live in the temperature range 15-20°C (Orcutt and Porter 1983), the temperature found in the epilimnion of many stratified temperate lakes. Further research into temperature selection by daphnids has indicated that when temperature is the only variable, *D. pulex, D. carinata,* and *D. middendorffiana* move to temperatures in the range 18-21°C (Wong 2002). The epilimnion of a temperate lake in summer would be an ideal habitat for *Daphnia* if temperature were the only factor controlling population growth.

In the epilimnion of clear lakes, resident planktivorous fish can see and easily capture *Daphnia.* To maintain a population in a lake with fish predators, *Daphnia* must adapt either by avoiding predation (Stich and Lampert 1981; Zaret and Kerfoot 1975) or by increasing their reproduction rate (Machacek 1993). Both of these strategies are seen in Lake Constance, Germany, here *D. galeata* remain in the warm epilimnion and high food, offsetting mortality from fish predation with higher reproduction, whereas *D. hyalina* avoid predation by exhibiting nocturnal diel vertical migration (DVM), residing in the dark, cold bottom layers of the lake in during the day and migrating to the warmer upper layers at dusk (De Meester 1994; Gliwicz 1986; Stich and Lampert 1981; Vos et al. 2002). *Daphnia* populations that have DVM reduce their predation by fish but consequently reduce their population growth by spending a part of each day in the cooler layer of

a lake. *Chaoborus*, phantom midge larvae, is an other predator of *Daphnia* (Havel and Dodson 1984; Tollrian 1995). *Chaoborus* also demonstrate DVM to avoid fish predation but these phantom midge larvae can also tolerate anoxic conditions found in the hypolimnion of meso-eutrophic lakes and come to epilimnion at dusk to feed on *Daphnia* and other zooplankton (Haney et al. 1990).

Food with inhibitory or toxic effects (Haney et al. 1994) also represent a potential cost when encountered by *Daphnia*. *Microcystis aeruginosa*, characteristic of eutrophic (Graham et al. 2006), but also mesotrophic and oligotrophic lakes (Haney and Ikawa 2000), produces the hepatotoxin (Carmichael 1994) microcystin a metabolite that is harmful to fish, livestock and humans (Blaha 1999; Robillot 2000; Falconer 2001; Oudra 2001; Vasconcelos 2001; Rao 2002). *M. aeruginosa* has also had deleterious effects on zooplankton, reducing reproduction (Ferrao-Filho et al. 2000) and clearance rates of *Moina* (Manish et al. 2001) and *Daphnia* (DeMott 1999). Neonates hatched from *Daphnia galeata* ephippia from recent sediment layers of Lake Constance, Germany, had higher growth rates when fed *M. aeruginosa* than the neonates hatched found from the older sediment layers preceding eutrophication in the lake (Hairston et al. 2001). These findings suggested that in a relatively short span of 50 years, D. *galeata* had undergone a genetic change becoming more resistant to the cyanobacteria.

Thus, selection of the optimal spatial distribution is a critical aspect of the ecology of zooplankton such as *Daphnia*. Fretwell and Lucus (1970) introduced the Ideal Free Distribution (IFD) model to describe habitat selection and predict the spatial distribution of birds colonizing a new environment. The IFD model

states that if a bird species has a choice of breeding sites within a habitat, the most ideal locations will be utilized by the first arrivals. As more birds occupy the most suitable habitat, later arriving individuals settle in less ideal habitats as the best locations become more crowded and resources become limited. The IFD model is also a useful model for predicting the distribution of *Daphnia* populations in a lake (Kessler and Lampert 2004b; Lampert 2005; Larsson 1997).

Assumptions of the IFD model include 1) all individuals in a population have identical reproductive fitness, 2) each individual has the freedom to choose its habitat site, and 3) suitable habitat can be easily assessed by scientific observers. Within most populations of animals, reproductive fitness and the ability to select an ideal habitat varies between individuals. However, *Daphnia* is a especially well-suited to test for IFD because its parthenogenic reproduction limits the individual variation within a population since genetic recombination does not occur during parthenogenesis and daughters are clones of their mothers (Hebert and Ward 1972). Habitat selection by *Daphnia* can be rapid as they quickly migrating layer from one layer to another. Additional attributes of *Daphnia* include their small size, short reproductive period, and ability to respond to contrasting food and temperature conditions in a model lake system (Calaban and Makarewicz 1982; Dawidowicz and Loose 1992a; Kinder 1995). Even columns as small as 30 cm can simulate the water column of a lake and the behavior of a *Daphnia* population can be assessed visually (Wong 2002).

In an experiment by Larsson (1997) *Daphnia pulex* distributed horizontally in accordance with the predictions of the IFD model. Single daphnids in a tank

always moved to the site of highest food concentrations. However, when 100 daphnids were placed in the same conditions, distributions observed were proportional to the density of *Daphnia* as predicted by the IFD model with most, but not all, of the animals going to the area of highest food concentration. In more complex natural systems, however, multiple costs such as predators, temperature, and food location interact causing deviations from the IFD model.

To address population distributions in complex habitats, Tyler and Gilliam (1995) developed the Ideal Free Distribution with Costs (IFD with Costs) model for predicting the distribution of fish in a stream by examining the variations in water velocity and food availability as possible costs. The IFD with Costs model has been recently applied to *Daphnia* in mesocosms simulating conditions in Lake Constance where high levels of chlorophyll occur in the hypolimnion. Lampert, McCauley and Manly (2003) studied the distribution of *Daphnia hyalina x galeata* in the 8 m high Plön plankton towers where they varied the temperature of the hypolimnion. Food additions to the bottom layer made the hypolimnion a more ideal environment for the daphnids than the warmer, foodless upper layer. The IFD with Costs model predicted that fewer animals would reside in the food-rich hypolimnion as the cooler temperatures increase the cost of living in the hypolimnion by reducing the metabolic rate, resulting in lower rates of growth and reproduction. Experimentally, the predicted IFD distribution was supported when 80% of the population was found in a hypolimnion that was only 2°C cooler than the epilimnion, but only 45% of the population was found in a hypolimnion that was 10°C cooler than the epilimnion. The interaction of population density and

temperature "costs" were examined in another plankton tower study (Lampert 2005). As the density of *D. pulicaria* increased over time, the population distribution changed from a narrow band around low food at the upper edge of the hypolimnion to a 3 m wide band extending into the hypolimnetic algal maximum, indicating that *Daphnia* avoided the high food region because of cold temperatures until the population density increased as predicted by the IFD with Costs model.

My study examines the vertical distribution responses of three *Daphnia* species in relation to the predictions of the IFD with Costs model. Previous experiments on vertical distribution using the IFD with Costs model used a deep water algal maximum (Kessler 2004; Kessler and Lampert 2004a; Lampert et al. 2003). In contrast, my investigation examines whether the deleterious cyanobacterium *M. aeruginosa* in the warm epilimnion represents a cost to the Ideal Free Distribution.

D. galeata, D. hyalina, and D. ambigua, were selected because of their distinct life histories and contrasting vertical distributions in nature and presumed different histories of exposure to cyanotoxins, temperature, and predator kairomones. The experimental *Daphnia galeata* were isolated from a non-migratory population (Lake Constance, Germany) that remains in the epilimnion and compensates for high predation losses by reproducing rapidly (Stich and Lampert 1981). *D. hyalina* were also isolated from Lake Constance, Germany, by W. Lampert, where they undergo pronounced migrations (Stich and Lampert 1981) as well as in many other lakes (De Meester et al. 1995; De Meester and Weider

1999; Hall 1964; Kessler 2004; Kessler and Lampert 2004b; Lampert et al. 2003; Spaak and Ringelberg 1997; Van Gool and Ringelberg 1997; Winder et al. 2004; Zaret and Suffern 1976). Stich and Lampert (1981) observed a DVM of 30 m by Lake Constance *D. hyalina* in July, 1977, but no noticeable migration in the population of *D. galeata* in Lake Constance during the same period. *D. hyalina* had reduced reproduction but maintained a stable population by avoiding fish predation through DVM. A third, non-migratory species, *D. ambigua*, was collected from Barbadoes Pond, Madbury, NH, that also has a migratory population of the phantom midge larvae, *Chaoborus punctipennis* (Haney et al. 1990), are predators on *D. ambigua* (Hanazato 1991; Hebert and Grewe 1985). Recent studies in Barbadoes Pond, NH, indicated that 86% of the *D. ambigua* population remained below the thermocline (unpublished data), minimizing contact with *Chaoborus* larvae. Similarly, in Aziscoos Lake, Maine, the *D. ambigua* population remained below the thermocline from June to August 1961 and 1962 at both noon and evening sampling (Tappa 1965).

Experiments were conducted to determine whether *M. aeruginosa* alter the IFD of three *Daphnia* species and whether deviations from the IFD are related to the fitness costs associated with cyanobacteria (i.e. growth rate and egg production). Cyanobacteria costs were first examined with experiments to determine the inhibitory effects of *M. aeruginosa* on the clearance and feeding rates of the three *Daphnia* species. I also conducted optical bioassay experiments to examine how specific aspects of the feeding mechanisms (thoracic beats and postabdominal food rejections) of the *Daphnia* species were influenced by cyano-

bacteria. Next, beaker feeding experiments were performed to ascertain the effect of *M. aeruginosa* on the fitness parameters of *Daphnia* without a spatial refuge. Two sets of column experiments were then performed to examine the distributional responses of the three *Daphnia* species in a simulated stratified lake, where a cold, food-poor hypolimnion provided a spatial refuge from the cyanobacteria in the warm epilimnion. The first columns tests examined the short-term (6-h) habitat selection patterns of animals in the same preconditioned state. A second, column experiment of longer duration (3 day) measured the fitness costs (egg production, lipid accumulation and growth) of the three *Daphnia* species given potentially available spatial refugia. Population distributions and temperature exposures were compared with fitness responses with and without a spatial refugium. These comparisons were used to assess the costs of deviations from the ideal free distribution in the high food and warm epilimnion. Species specific cost tradeoffs are discussed in relation to the contrasting natural history and ecology of each *Daphnia* species.

CHAPTER 2

METHODS

Culture maintenance

All animals were maintained in 1 L containers of aerated well water (pH=7.8, <0.5 mg L^{-1} nitrite, 34 mg L^{-1} sodium, 39 mg L^{-1} chloride), kept in a temperature controlled room (20 ±1°C). The light regime was 14 h low light intensity (5 µmol m^{-2} s^{-1}) to 10 h dark (<0.1 µmol m^{-2} s^{-1}). Clones of *D. hyalina* and *D. galeata* were originally isolated in about 1980 from Lake Constance, Germany, by W. Lampert of the Max Plank Institute, Plön, Germany. *D. ambigua* were collected from Barbadoes Pond, Madbury, New Hampshire, in February, 2003. To begin new cultures, age cohorts were begun by removing 1-2 day old neonates to a new culture container.

All *Daphnia* were fed green algae, *Scenedesmus obliquus*, strain #2630 obtained from the University of Texas Culture collection (UTEX) observed in groups of four cells when counted at 100X magnification with a compound light microscope. Batch cultures of *S. obliquus* in ASM-1 media (Mclaughlin and Gorham 1961) were maintained in 500 ml tubes under constant light intensity (15 µmol m^{-2} s^{-1}) and temperature (20 ±1°C). Dry weight of a single *S. obliquus* cell was measured by weighing a filter membrane (0.2 µm GTTP Isopore membrane filter) onto which 5 ml of stock of known concentration had been filtered through the membrane and dried for 24 h at 60°C. Average dry weight of *S. obliquus* was

88.4 ± 0.2 pg cell^{-1} with half of the dry weight estimated to be carbon (Geller 1975). In all experiments with *S. obliquus*, the 1 g C L^{-1} was equivalent to 2.3 x 10^4 cell ml^{-1}. *M. aeruginosa*, strain #2385, was obtained from UTEX and observed to be individual cells under 400X magnification. Batch cultures of *M. aeruginosa* were maintained under the same temperature and light conditions as *S. obliquus*. The average dry weight of *M. aeruginosa* was 8.9 pg cell^{-1} (Trubetskova and Haney 2000) with 1 g C L^{-1} equal to 2.3 x 10^5 cell ml^{-1} of *M. aeruginosa*. For maintenance of *Daphnia cultures, S. obliquus* was added daily in excess of 1.0 mg C L^{-1}, the incipient limiting concentration for growth (Lampert 1977). Concentrations of stock algae were measured with a hemacytometer, counting a minimum of 100 cells.

Clearance rates with *S. obliquus* and *M. aeruginosa*

Clearance rates for each *Daphnia* species were measured from experiments conducted over a 3 h period at 25 °C. For each treatment, 10 daphnids were placed in a 125 ml flask containing 50 ml well water and either *S. obliquus* (1 g C L^{-1}) or *Microcystis aeruginosa* (1 g C L^{-1}). Food suspensions were also added to control flasks that did not contain *Daphnia*. Flasks were placed on an orbital shaker at 44 revolutions min^{-1} (gyratory water bath shaker, Model G76, New Brunswick Scientific) to prevent the algae and cyanobacteria from settling to the bottom. Treatments were repeated in triplicate for each species and control.

To determine a change in algal concentrations, chlorophyll *a* content of pre-experiment suspension ($Fluor_{init}$) and post-experiment suspension ($Fluor_{final}$) was measured with an Aquafluor Handheld Fluorometer (Turner Designs, Sunnyvale, CA). Clearance rates (CR) for each flask were calculated according to Peters (1984) using the fluorescence values for chlorophyll *a* in the following equation:

$$CR\ (ml\ ind^{-1}\ h^{-1}) =$$

$$[\ln(Fluor_{init})-\ln(Fluor_{final})\ x\ vol\ (ml)] \div (time\ (h)\ x\ no.\ \textit{Daphnia})$$

where volume is the quantity of liquid in the flask and time is the duration of the experiment. Clearance rates were calculated for the control flasks using the average number of individuals in each experimental flask. Control CR were subtracted from the experimental CR.

At the conclusion of the experiments, all animals were removed from the flasks and preserved in a 4% Formalin/4% sucrose solution (Haney and Hall 1973) to measure body length of the daphnids at a later time. Body lengths were measured from the top of the head to the base of the tail spine ±0.01 mm with the MetaMorph computer software and image capture system (Molecular Devices, Sunnyvale, CA, v 4.1). For each flask, a mean body length was calculated from all 10 animals in the replicate. Clearance rates (CR_{corr}) for both *D. hyalina* and *D. galeata* were corrected to a standard body length (BL_{std}) of 1.5 mm and *D. ambigua* to a standard body length of 1.0 mm using the following relationship described by Burns (1969):

$$CR_{corr} = Intercept_{flask} * (BL_{std})^{2.38}$$

Optical bioassay of feeding mechanisms

Changes in thoracic beats of the three *Daphnia* species were measured with a bioassay experiment as described by Haney et al. (1995) with video recording system developed by Sasner & Haney (unpublished), later described by Lampert and Brendelberger (1996), and subsequently illustrated by Wong (2002). Thoracic beats are a useful surrogate measurement for feeding activities of *Daphnia* since filtering rates are correlated with thoracic beat rates (Balf 1988). Pre-experimental conditions were established by placing three *Daphnia* of each species in 50 ml of *S. obliquus* (1.0 mg C L^{-1}) for 30 min. For each assay, one animal was affixed by the back of the carapace to a thin line of petroleum jelly in the middle of a 15 ml petri dish and the experimental treatments were recorded on video tape for analysis after all replicates were completed. For the initial measurements, 10 ml of a *S. obliquus* (1.0 mg C L^{-1}) suspension of was added to the dish. The experiment was recorded after 30 s of acclimation and continued for 5 min. Next, the *S. obliquus* suspension was removed by pipette and replaced with a 10 ml suspension of *M. aeruginosa* (1.0 mg C L^{-1}). These experimental conditions were recorded on video tape for 5 min after a 30 s acclimation period. The final recording consisted of a 5 min recovery period for the animal in a *S. obliquus* algae suspension after the *M. aeruginosa* suspension was removed. The video tape was played back on a screen with a light sensor placed where the end of one of the filtering appendages is moving back and forth on the

screen. The analog to digital converter (MacLab, Chart 5.2 software program (ADInstruments, Colorado Springs, CO, USA) converts the light signal changes caused by the beating thoracic appendage to a sine wave graph (Fig. 1). The total number of post-abdominal rejections were counted and represented on the graph by irregular fluctuations (Fig. 1, indicated by arrows labeled "PAR"). From the sine wave graph, the total number of thoracic beats min^{-1}(TB) was calculated as were the rates of TB between PAR (Fig. 1 indicated by brackets labeled A-E). The food collection "cost" of post abdominal rejections, considered to be the thoracic beats lost as a result of food rejections, was calculated as:

"Cost" of PAR on TB = [Average (TB between PAR)] – Mean Total TB.

Beaker feeding experiments without a spatial refuge

Feeding experiments were conducted with 5-day old *Daphnia* of each species, an appropriate age to observe the growth and first egg production since the first brood of eggs is produced at approximately 8 d at 20°C (Lundstedt and Brett 1991; As summarized by Lynch 1980). Treatments were repeated in triplicate for each species. To prevent size bias, all individuals used for pre-experimental measurements and experimental treatment were selected at the same time. The first nine individuals were removed from the cohort container by pipette and placed into nine separate 40 ml containers. Animals were then distributed evenly in the same way until each contained 5 animals. Pre-experimental measurements of lipid index, egg counts, length and dry weight

were assessed for individuals in three of the containers. The lipid index, a measure of nutritional state developed by Holm and Shapiro (1984), has the following four categories: 0 = <10 lipid droplets, 1 =10- 49 lipid droplets, 2 = 50-99, and 3 = ≥100 lipid droplets. Number of eggs or embryos in the brood pouch was counted under a compound light microscope at 40X magnification and expressed as the egg ratio, or number of eggs female^{-1} (Edmondson 1968). Five *Daphnia* were placed in pre-weighed aluminum weigh boats, oven-dried at 60°C for 24 hr, and then weighed on a microbalance (Cahn Microbalance C-31, ±0.1 μg). Weights at the beginning of the experiments ($weight_{init}$) were compared with weights at the conclusion of the experiments ($weight_{final}$) to determine growth rate over the duration of the experiment. Growth rate (GR) was calculated from the following equation:

$$GR = (\ln(weight_{final}) - \ln(weight_{init})) \div time$$

where time is the duration of the experiment in days.

For each 5-d experiment, five daphnids were placed in a beaker containing 100 ml filtered well water (Whatman Glass Microfiber Filters, Type GF/C, pore size ≈ 1.2 μm). The concentration of food in each beaker was 1.0 mg C L^{-1} of either *S. obliquus* or *M. aeruginosa*. Daphnids were transferred daily by pipette to beakers with fresh food suspensions. The experimental animals were cultured in a temperature controlled room (20 ±1°C) in constant light (7 μmol m^{-2} s^{-1}). No stratification was set up in the beakers, so there was no refuge for the daphnids from the *M. aeruginosa*. At the termination of the experiment, the body length, lipid count, and egg count were measured for each individual. All indi-

viduals from a single beaker were dried and weighed in the same manner as the pre-experiment animals to assess the final dry weight.

Costs were estimated as the impact of *M. aeruginosa* on each fitness parameter measured. For egg ratio, length, and growth rate, the costs were calculated by dividing the value from the *M. aeruginosa* treatment by the *S. obliquus* treatment and subtracting the resulting proportion from 100%. For the lipid index cost, the average proportion of change was first calculated for both food treatments individually by subtracting the initial lipid index from the final lipid index and dividing by the initial lipid index. The total cost was then calculated by subtracting the cost of *M. aeruginosa* from the cost of *S. obliquus*

.

Six hour habitat selection patterns in columns with refuge

The spatial response of *Daphnia* to *M. aeruginosa* was recorded as the depth distribution of 10 animals in small columns over a 6 h experiment under full light conditions. The simulated lake system consisted of four attached Plexiglas columns, each 30 cm x 4.25 cm x 3.25 cm as used by Kinder (1995) and Wong (2002) (Fig. 2). The columns were illuminated from above by halogen lights maintained at 21 μmol m^{-2} s^{-1} simulating the light intensity at 3 m in Barbadoes Pond (unpublished data) measured with a Li-Cor quantum sensor (Li-192SA, LI-COR Biosciences, Lincoln, NE). Well water for the control columns was filtered in the same manner as the beaker experiment. For columns with *D. galeata* and *D. hyalina,* fish conditioned well water (3 *Lepomis gibbosus* in 19 L) was filtered

through GF/C membranes to provide fish kairomones (De Meester and Weider 1999; Van Gool and Ringelberg 1997). For columns with *D. ambigua*, invertebrate predator conditioned well water (20 *Chaoborus punctipennis* L^{-1}) was filtered through GF/C membranes to provide predator kairomones slightly higher than the experimental concentration used by Boeing et al. (2005). *C. punctipennis* were collected from Old Durham Reservoir, Durham, New Hampshire, in October, 2005, and fed *Daphnia*. A temperature gradient or thermocline was established at 15 cm by the flow-through design of the columns (Fig. 2). The temperature of the water in the upper half of the columns was maintained between 20 and 22°C by the ambient temperature of the experimental environment. A recirculating cooler water bath (VWR Scientific, model 1150A, Niles, IL) constantly pumped cold water through the rear chamber of the Plexiglass column system to maintain the water in lower half of the columns at 8-10°C. To minimize any disturbances to the *Daphnia* behavior by compressor noise (Buchanan and Goldberg 1981), the cooler was kept outside the experiment room but connected to the experimental system by Tygon tubing.

After the temperature gradient was established, food was added to the epilimnion of the columns. Final concentrations were either 1.0 mg C L^{-1} of *S. obliquus* or 1.0 mg C L^{-1} of mixed food (0.5 mg C L^{-1} of *S. obliquus* and 0.5 mg C L^{-1} of *M. aeruginosa*). Concentrations of 0.5 mg C L^{-1} *M. aeruginosa* in a mixed food suspension caused reduced growth and reproduction similar to starvation in *D. magna* (Trubetskova and Haney 2006). After the food suspension was added, 10 daphnids of a single species were pipetted into the columns (Ta-

ble 1). To avoid bias and possible position effects, the order of treatment assignments were randomized across columns.

During the 6 h experiment, the temperature and daphnid depth location was measured every 15 min by direct observation by eye. The temperature was recorded at 5 cm from the surface, 15 cm from the surface, and 25 cm from the surface. The number of daphnids in each 5 cm vertical segment of a column was recorded. The experiment was repeated with different individuals three times for each species to allow for statistical comparisons. Temperature exposure for the population quartiles (Pennak 1943) was estimated from four parametric logistic non-linear regressions of temperature and depth (adj. r^2=0.995) with to the following equation (SigmaStat 3.1, Systat Software, Inc.):

Temperature (^{o}C)= (a/(1+abs(depth (cm)/c)^b)+d

where a=asymptotic maximum, b=slope parameter, c=value at inflection point, d=asymptotic minimum. For example, the temperature curve in the first replicate with *D. ambigua* had the following variables: a=14.75, b=6.30, c=16.83 and d=7.48.

Fitness costs of three days in columns with spatial refuge

Experiments lasting 3 days were conducted in the same simulated system as used in the 6 h experiments with the same food and temperature conditions as in the previous column experiments. However, whereas the 6 h experiments were conducted with constant light, the 3 day experiments utilized a diel light cy-

cle including a sunrise and sunset To simulate a natural sunset and sunrise, a computer program (Basic language) used by Schloss (1997) was modified by Dr. R. Spanke to adjust for increased processing speed (Appendix 1: Light Control Program for Basic). The light program incrementally increased and decreased the lighting system from full power (21 μmol m^{-2} s^{-1}), representing full sunlight, to minimal power (<0.1 μmol m^{-2} s^{-1}) representing darkness. The light levels were changed on intervals of 60 sec and the values simulated a 24 h period including a sunset and a sunrise. The sunset simulated the actual rate of changes in light recorded during a sunset on April 10, 1998 in Barrington, NH, with the time from full light to no detectable light being 37 min. The simulated sunrise was a sunset run in reverse. The length of the daylight was 12 h, 35 min, to simulate September 15, 2004 at 47°39′N 9°19′E, the coordinates of Lake Constance, Germany (US Naval Observatory, http://aa.usno.navy.mil/data/docs/RS_OneDay.html). The date was chosen because both *D. hyalina* and *D. galeata* demonstrated the greatest amplitude of DVM in Lake Constance at that time of year (Stich and Lampert 1981). The lights were cycled through three sets of sunsets over the course of 72 h to simulate a three day period in mid September.

Water temperature and daphnid vertical distribution were measured 2-6 times in each light and dark period. During dark periods, the location of each daphnid was observed with a red filtered light to limit disturbance from illumination (Scheffer et al. 1958 as described by Dawidowicz and Loose 1992b; Smith and Baylor 1953). Once each day, a 1 ml sample of water was taken from both

the epilimnion and hypolimnion section of each column to determine monitor the algal and cyanobacterial concentrations and food concentrations were adjusted to initial dilutions.

Temperature exposure of population quartiles were calculated as in previous experiment. After 3 d, the animals were removed and corresponding body length, lipid index, and egg counts were measured for each individual as previously described for the beaker experiments. Costs of each parameter were calculated as in the beaker feeding experiments.

Statistical Analysis

Tests for statistical significance were performed with the SigmaPlot 9.01 and SigmaStat 3.11 software (Systat Software, Inc., Richmond, CA, USA). T-tests were used to compare within species differences of all parameters measured. One-way ANOVA analyzed with Tukey post-hoc tests were conducted on the data to compare differences between species. Data from the optical bioassay experiments were analyzed with repeated measures one-way ANOVA tests. Unless otherwise stated, the threshold for statistical significance was $p \leq 0.05$.

CHAPTER 3

RESULTS

Clearance rates with *Microcystis aeruginosa* and *Scenedesmus obliquus*

The clearance rate of *D. hyalina* fed *S. obliquus* for 3 h was 0.69 ml ind^{-1} h^{-1} (Table 2) and was slightly more than twice the clearance rate of *D. hyalina* fed *M. aeruginosa* (0.31 ml ind^{-1} hr^{-1}, Table 2, Fig. 3). *D. galeata* and *D. ambigua* tended to clear the water of *S. obliquus* nearly twice as fast as of *M. aeruginosa* (Fig. 3). However, differences between the rates were not statistically significant.

Optical bioassay of feeding mechanisms

The total thoracic beat rate of all animals was not reduced by the addition of *M. aeruginosa* (Table 3). *D. hyalina* had approximately the same thoracic beat rate when feeding on either *S. obliquus* (386.0 and 403.2 beats min^{-1}) or *M. aeruginosa* (385.5 beats min^{-1}, Table 3). Although none of the differences were not statistically significant ($p>0.05$), *D. ambigua* had reduced thoracic beat rate when feeding on *M. aeruginosa* as did *D. galeata*. Both *D. hyalina* and *D. galeata* increased thoracic beat rates during the recovery period while *D. ambigua* reduced its thoracic beat rates during the recovery period. Within species variations were not statistically significant for the cost of post abdominal rejec-

tions on thoracic beats, although *D. hyalina* and *D. galeata* had a greater cost and *D. ambigua* had a reduced cost fed *M. aeruginosa* (Fig. 4).

Fitness parameters in *M. aeruginosa* and *S. obliquus* without a refuge

During a 5 d period differences between treatments were observed for animals fed *S. obliquus* and *M. aeruginosa* in some, but not all, parameters measured (Table 4). *D. ambigua* had significantly more eggs per female (1.93 ± 0.07 eggs female^{-1}, Table 4) when fed *S. obliquus* than when fed *M. aeruginosa* (Fig. 5, $p<0.001$). Similarly, both *D. hyalina* and *D. galeata* had a higher egg ratios when fed *S. obliquus* although not statistically significant (Table 4, Fig. 5).

The lipid index decreased significantly in all daphnids fed *M. aeruginosa* (Fig. 6); D. *galeata* had the greatest cost on lipids, 390%, when fed *M. aeruginosa* (Table 4). Within species, the proportional increase in length was statistically significant for *D. galeata* (Table 4, Fig. 7, $p=0.006$). Although not statistically significant, the proportional length increase was greater for both *D. hyalina* and *D. ambigua* fed *S. obliquus* (Table 4, Fig. 7).

The growth rate of *D. hyalina* fed *S. obliquus* (0.19 day^{-1}, Table 4) was significantly greater than the growth rate of *D. hyalina* fed *M. aeruginosa* (Fig. 8, $p=0.002$). *D. galeata* and *D. ambigua* fed *M. aeruginosa* tended to grow slower than animals fed *S. obliquus* (Fig. 8) although the rates did not differ significantly (Table 4).

Distribution patterns over 6 h in columns with refuge

D. ambigua had the most distinctive vertical distribution pattern of the three *Daphnia* species. The 50-percentile depth of *D. ambigua* over 6 h in the 30 cm columns was between 21-23 cm with either *S. obliquus* or *M. aeruginosa* and either with or without *C. punctipennis* kairomones (Fig. 9), placing the mean population depth below the thermocline in water between 11°C and 14°C (Fig. 10). In contrast, the average 50-percentile depths of *D. hyalina* and *D. galeata* were 9.5 cm and 11.1 cm, respectively. Both *D. hyalina* and *D. galeata* remained in the warmer epilimnion when fed either *M. aeruginosa* or *S. obliquus* (Fig. 9). In all conditions except when fed *S. obliquus* in water without fish kairomones, 75% of *D. hyalina* remained above the thermocline (Figs. 9 & 10) at all times. At all times, 75% of *D. galeata* remained at or above the thermocline (Figs. 9 & 10). Between species, daphnids in water without kairomones and fed *S. obliquus* were distributed over the widest range of depth and temperature (Figs. 9 & 10).

Distribution patterns over 3 d in columns with refuge

The vertical distribution differed between species over the course of the 3 day experiment. The median depth of *D. ambigua* in both light and dark conditions was 23.1 cm, below the 15 cm thermocline (Fig. 11). The *D. ambigua* experienced a median temperature of 14°C (Fig. 12) in the hypolimnion. During the 3 day experiments, 75% of both *D. hyalina* and *D. galeata* were in the epilimnion

whether fed *M. aeruginosa* or *S. obliquus* (Fig. 11). The range of the quartile depths and temperature for both *D. hyalina* and *D. galeata* were all above the thermocline throughout the experiments (Figs. 11 & 12). The depth and temperature range for *D. galeata* (Figs. 11 & 12) was similar to that of *D. hyalina* although the lowest part of all but the *M. aeruginosa* and fish kairomones treatment extended into the thermocline. The opposite was observed in the columns of *D. ambigua* (Figs. 11 & 12). The mean depth for *D. ambigua* in all conditions was below the thermocline (Fig. 11) with the mean temperature experienced between 11-14°C (Fig. 12). In all experiments, the daphnids moved down in the water column in dark conditions (Figs. 11 & 12), although that trend was not statistically significant within species. Across species, the depth range of daphnids was smaller when the food source contained *M. aeruginosa* (Figs. 11 & 12).

Fitness costs of 3 d in columns with spatial refuge

Overall, Daphnia tended to produce fewer eggs in the columns with *M. aeruginosa* despite the presence of a refuge, although the differences were not statistically significant (Table 5). Differences were most pronounced for *D. hyalina*, which produced 75% fewer eggs in the columns with 50% *Microcystis*.

Both *D. hyalina* and *D. galeata* had lipid index values significantly reduced when fed *M. aeruginosa* with a cost of 113% and 90%, respectively (Table 5). *D. ambigua* had a reduction in the lipid index in both treatments (Fig. 13), but the

cost of feeding on *M. aeruginosa* was 35% greater than the *S. obliquus* treatment (Table 5).

The cost of feeding on 50% *M. aeruginosa* was not as dramatic for the proportional change in length. For *D. hyaline*, the cost of feeding on *M. aeruginosa* was 8% (Table 5). For both *D. galeata* and *D. ambigua*, the cost of feeding on *M. aeruginosa* was only 5% (Table 5).

The growth rate of all *Daphnia* was reduced when feeding on *M. aeruginosa* (Fig. 14). The cost was greatest on *D. hyalina*, 138% (Table 5). The cost of feeding on *M. aeruginosa* on the growth rate of *D. galeata* was 76% and 65% for *D. ambigua* (Table 5).

CHAPTER 4

DISCUSSION

Comparison of natural and experimental spatial distributions

Vertical distributions of the *Daphnia* studied were consistent with the expected distributions for two of the three species tested. *D. ambigua* consistently remained below the thermocline despite the cold temperatures and low food (Figs. 9 & 11). This meta-hypolimnetic distribution conforms to those observed in the field (Tappa 1965). *D. galeata* remained in the epilimnion (Figs. 9 & 11) despite fish kairomones and food that had negative effects on its growth and reproduction (Tables 4 & 5). The epilimnetic distribution of *D. galeata* was consistent with field observations in Lake Constance (Stich and Lampert 1981; Stich and Lampert 1984). Surprisingly, *D. hyalina* also remained in the epilimnion and showed no major diel migration during these experiments which differs markedly from its behavior in Lake Constance (Stich and Lampert 1981) and other European lakes (Ringelberg et al. 1991). The lack of migration by *D. hyalina* in the columns could be attributed to some natural condition missing in the columns. For example, inadequate strength of predator kairomones might not provide the necessary stimulus for enhanced phototaxis (Ringelberg and Van Gool 2003). However, De Meester and Weider (1999), found that a fish concen-

tration of only 1 fish per 20 L, approximately one-third of my experimental level, evoked distributional movements in *D. hyalina.* It is possible that the kairomones degraded over the course of 3 days; however, no shift in depth distribution was seen over time to support this condition. Another possibility is that *D. hyalina* are adapted to respond to a particular fish species such as perch (*Perca fluviatilis*) that occur in Lake Constance and not to the sunfish (*Lepomis gibbosus*) used in this study. There is some support for this possible cause, considering a recent study demonstrated that perch and stickleback elicit different life history responses in *Daphnia galeata* (Weber 2003). Another possible factor explaining why *D. hyalina* did not move into the hypolimnion could that the clone of this species has been in culture for over 20 years and may have lost the adaptation for migration or, by chance, might have been a non-migrating phenotype when first put into culture (De Meester and Weider 1999). A recent study by Gliwicz et al. (2006) reveals another potential cause for the unexpected distributional pattern of *D. hyalina.* These authors report that the density of the *D. hyalina* population can alter its behavior, whereby it can be induced to migrate in columns only at high population densities.

M. aeruginosa as a cost to spatial distribution

A major goal of this study was to determine if *M. aeruginosa* would alter the spatial distribution of *Daphnia* and thus represent a cost to the IFD model. Consistent with the IFD with Costs model, *D. ambigua* did shift its distribution in

the presence of *M. aeruginosa* to move to a habitat with negative attributes (cold water and low food concentration). In the presence of *M. aeruginosa*, the vertical distribution of *D. ambigua* was deeper (Figs. 9 & 11) and thus subjected the animals to colder water. In addition to its distributional response to *M. aeruginosa*, D. *ambigua* also reduced its clearance rate when feeding on *Microcystis* (Fig. 3). The fitness costs of exposure to the cyanobacteria were reductions in egg ratio (Fig. 5), lipids (Fig. 6), and growth (Figs. 8 & 14).

In contrast, the spatial distributions of *D. hyalina* and *D. galeata* were not influenced by *M. aeruginosa.* The response of both species was consistently to remain in the warmer epilimnion (Figs. 10 & 12) despite the presence of the harmful cyanobacteria. Despite fitness costs due to *M. aeruginosa* including reductions in egg ratio (Fig. 5), lipids (Figs. 6 & 13), and growth (Figs. 8 & 14) *D. hyalina* and *D. galeata* appeared to rely solely on feeding behavior (increased food rejection) to reduce the ingestion of the *Microcystis.*

These contrasting behaviors in the three species suggest that *Daphnia* may have evolved very different adaptations to ameliorate the impact of living in an environment with an abundance of ingestible cyanobacteria. The Lake Constance *Daphnia* appear to utilize feeding mechanisms to protect against eating cyanobacteria, whereas *D. ambigua* employ a combination of feeding and spatial avoidance. Of course, it would be useful to test whether these species exhibit other adaptations not examined here when confronted with cyanobacteria containing different chemical deterrents against herbivores.

TABLES AND FIGURES

Table 1. Matrix of conditions used in the four columns during 6-h experiments. *Scenedesmus obliquus* (green alga) and *Microcystis aeruginosa* (cyanobacterium) were added to the epilimnion only. The total amount of food in carbon equivalent was 1 mg C L^{-1}. The predator kairomones for both *Daphnia hyalina* and *D. galeata* was from *Lepomis gibbosus* (pumpkinseed sunfish). The predator kairomone for *D. ambigua* was from *Chaoborus punctipennis* (phantom midge larvae).

	Condition 1	**Condition 2**	**Condition 3**	**Condition 4**
Food	100% *S. obliquus*	100% *S. obliquus*	50% *S. obliquus* 50% *M. aeruginosa*	50% *S. obliquus* 50% *M. aeruginosa*
Water	Well	Predator kairomones	Well	Predator kairomones

Table 2. Clearance rates (CR) of three *Daphnia* species fed 1 mg C L^{-1} of either *Scenedesmus obliquus* or *Microcystis aeruginosa* for 3 h (n=3 replicates). Values in parenthesis indicate standard error. P-values were obtained from student t-tests performed within species results.

	CR (ml ind^{-1} h^{-1})				
	S. obliquus		*M. aeruginosa*		p-value
D. hyalina	0.69	(0.04)	0.31	(0.13)	**0.05**
D. galeata	0.73	(0.29)	0.40	(0.26)	0.44
D. ambigua	0.38	(0.08)	0.17	(0.06)	0.12

Table 3. Summary of results from optical bioassay. The feeding regime for each daphnid was 5 min in *Scenedesmus obliquus*, *Microcystis aeruginosa*, and then *S. obliquus*. Total thoracic beat rate, post-abdominal rejections, and "cost" of PAR, or thoracic beats reduction due to post-abdominal rejections. Mean value for each parameter is listed (n=3 replicates). Values in parentheses are standard errors. Statistical values were obtained from repeated measures one-way ANOVA between treatments within each species.

	Total Thoracic Beat (TB) Rate (beats min^{-1})						
	S. obliquus		*M. aeruginosa*		*S. obliquus*		p-value
D. hyalina	386.0	(12.3)	385.5	(12.2)	403.2	(24.0)	0.25
D. galeata	357.7	(9.9)	310.4	(16.7)	348.5	(29.1)	0.22
D. ambigua	403.2	(19.3)	386.0	(5.8)	351.8	(15.3)	0.07

	Post-Abdominal Rejections (PAR min^{-1})						
	S. obliquus		*M. aeruginosa*		*S. obliquus*		p-value
D. hyalina	1.4	(0.5)	2.9	(1.2)	3.2	(0.6)	0.44
D. galeata	3.8	(0.6)	4.8	(0.7)	3.1	(0.1)	0.29
D. ambigua	4.8	(1.1)	5.5	(1.0)	5.0	(1.5)	0.92

	TB Reduction by PAR (beats min^{-1})						
	S. obliquus		*M. aeruginosa*		*S. obliquus*		p-value
D. hyalina	2.7	(1.1)	9.2	(4.3)	7.2	(1.8)	0.14
D. galeata	-0.2	(4.0)	4.4	(1.0)	-3.2	(6.7)	0.60
D. ambigua	13.7	(8.8)	10.8	(3.3)	16.5	(10.8)	0.88

Table 4. Fitness parameters of three *Daphnia* species after 5 d of feeding either 100% *Scenedesmus obliquus* or 100% *Microcystis aeruginosa* without a refuge. Mean value for each parameter is listed (n=3 replicates). Values in parentheses indicate standard error. P-values were obtained from student t-tests performed on results within species; Bold values indicate statistical significance ≤ 0.05.

	Egg Ratio (eggs female $^{-1}$)					
	S. obliquus		*M. aeruginosa*		p-value	Cost of feeding on *M. aeruginosa*
D. hyalina	0.60	(0.35)	0.33	(0.07)	0.49	44%
D. galeata	0.53	(0.07)	0.40	(0.06)	0.21	25%
D. ambigua	1.93	(0.07)	0.00	(0.00)	**<0.001**	100%

	Change in lipid index					
	S. obliquus		*M. aeruginosa*		p-value	Cost of feeding on *M. aeruginosa*
D. hyalina	0.73	(0.12)	-1.60	(0.07)	**<0.001**	140%
D. galeata	0.67	(0.03)	-0.50	(0.00)	**<0.001**	390%
D. ambigua	0.09	(0.35)	-1.44	(0.00)	**0.01**	106%

	Proportional change in length					
	S. obliquus		*M. aeruginosa*		p-value	Cost of feeding on *M. aeruginosa*
D. hyalina	1.31	(0.05)	1.20	(0.04)	0.17	8%
D. galeata	1.09	(0.01)	1.01	(0.01)	**0.006**	7%
D. ambigua	1.73	(0.10)	1.56	(0.06)	0.22	10%

	Growth rate (day^{-1})					
	S. obliquus		*M. aeruginosa*		p-value	Cost of feeding on *M. aeruginosa*
D. hyalina	0.19	(0.02)	0.03	(0.00)	**0.002**	86%
D. galeata	0.04	(0.03)	0.00	(0.04)	0.41	96%
D. ambigua	0.15	(0.06)	0.04	(0.02)	0.17	72%

Table 5. Fitness parameters of three *Daphnia* species after 3 d of feeding in columns with a spatial refuge. The food given was either 100% *Scenedesmus obliquus* or 50% *Microcystis aeruginosa* and 50% *S. obliquus* in the epilimnion of a simulated lake (n=3 replicates). Values in parentheses indicate standard error. P-values are based on statistical analysis using a student t-test. Bold values indicate statistical significance ≤ 0.05.

Egg Ratio (eggs female $^{-1}$)					
	S. obliquus	*M. aeruginosa*	p-value	Cost of feeding on *M. aeruginosa*
D. hyalina	3.58 (1.06)	0.90 (0.45)	0.08	75%
D. galeata	2.04 (0.80)	0.95 (0.28)	0.27	53%
D. ambigua	1.27 (0.59)	0.73 (0.58)	0.56	42%

Change in lipid index				
	S. obliquus	*M. aeruginosa*	p-value	Cost of feeding on *M. aeruginosa*
D. hyalina	0.48 (0.27)	-0.71 (0.33)	**0.05**	113%
D. galeata	0.13 (0.28)	-1.13 (0.38)	**0.05**	90%
D. ambigua	-0.73 (0.41)	-1.33 (0.38)	0.34	35%

Proportional change in length				
	S. obliquus	*M. aeruginosa*	p-value	Cost of feeding on *M. aeruginosa*
D. hyalina	1.33 (0.05)	1.22 (0.08)	0.30	8%
D. galeata	1.32 (0.09)	1.25 (0.07)	0.50	5%
D. ambigua	0.97 (0.04)	0.92 (0.05)	0.52	5%

Growth rate (day^{-1})				
	S. obliquus	*M. aeruginosa*	p-value	Cost of feeding on *M. aeruginosa*
D. hyalina	0.15 (0.22)	-0.06 (0.07)	0.35	138%
D. galeata	0.21 (0.04)	0.05 (0.09)	0.17	76%
D. ambigua	0.21 (0.15)	0.07 (0.15)	0.55	65%

Table 6. The difference in "cost" of not having a spatial refuge on measured fitness parameters of feeding on *Microcystis aeruginosa* and *Scenedesmus obliquus* between refuge and no refuge experiments of each of the *Daphnia* species studied. A large positive number indicates a species did much better for that parameter when able to avoid *M. aeruginosa* by going to the hypolimnion of a column. A negative number indicates a species was not able to avoid the effect of *M. aeruginosa* in the simulated lake.

	Cost of not having a refuge			
	Eggs female^{-1}	Lipid index	Growth rate	Length
D. hyalina	-30%	27%	-52%	-1%
D. galeata	-28%	300%	21%	2%
D. ambigua	58%	71%	6%	5%

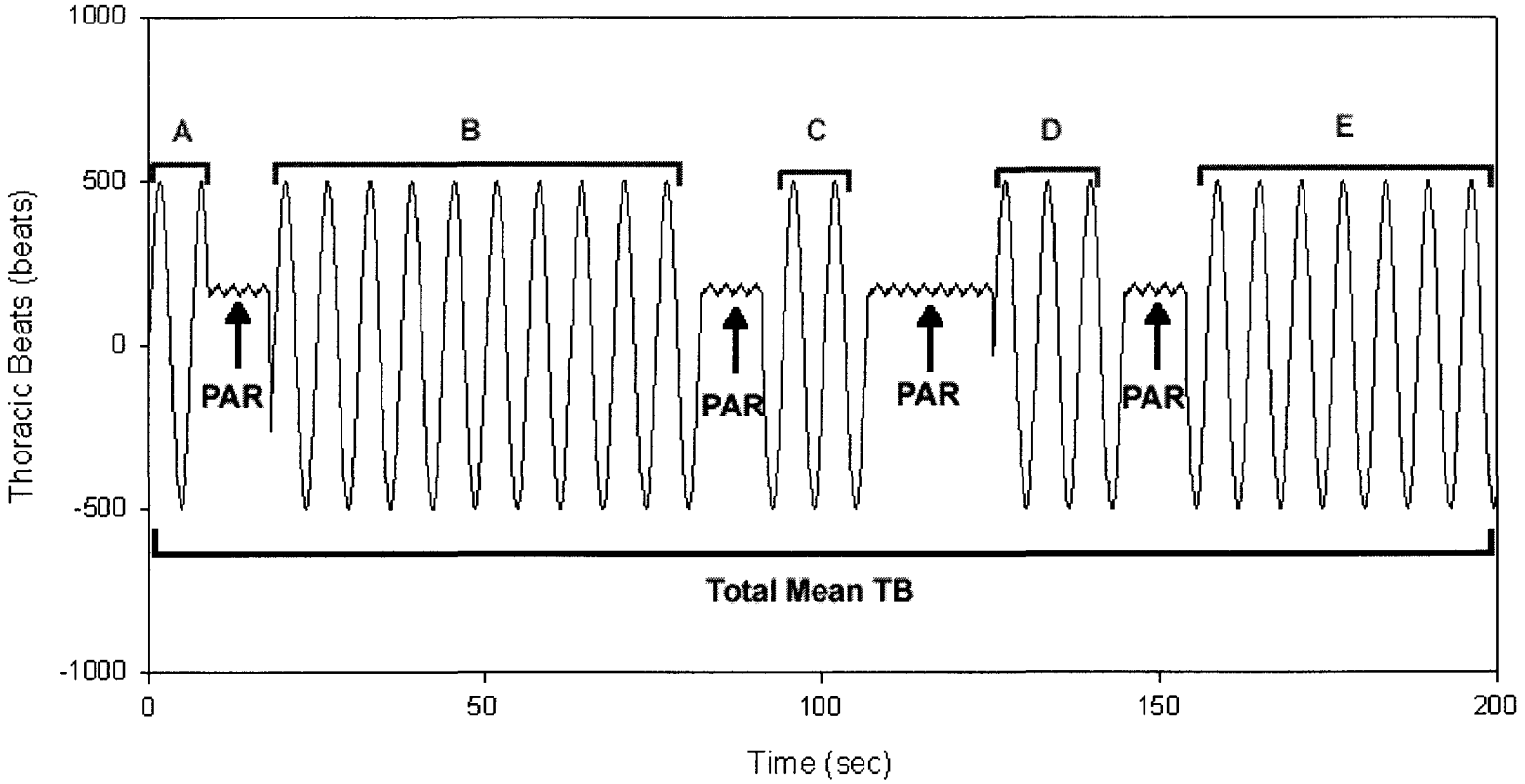

Figure 1. A sample graph created by Chart 5.2 program to illustrate the method used to estimate thoracic beat rate "cost". The total mean TB is the average thoracic beat rate for the entire 200 sec segment. The sections marked A, B, C, D, & E represent the rates of TB between post-abdominal rejections (PAR). The "cost" of PAR is the mean value of A, B, C, D & E minus the total mean TB. The equation is as follows:

"Cost" of PAR = [Average(A,B,C,D,E)] – Total Mean TB

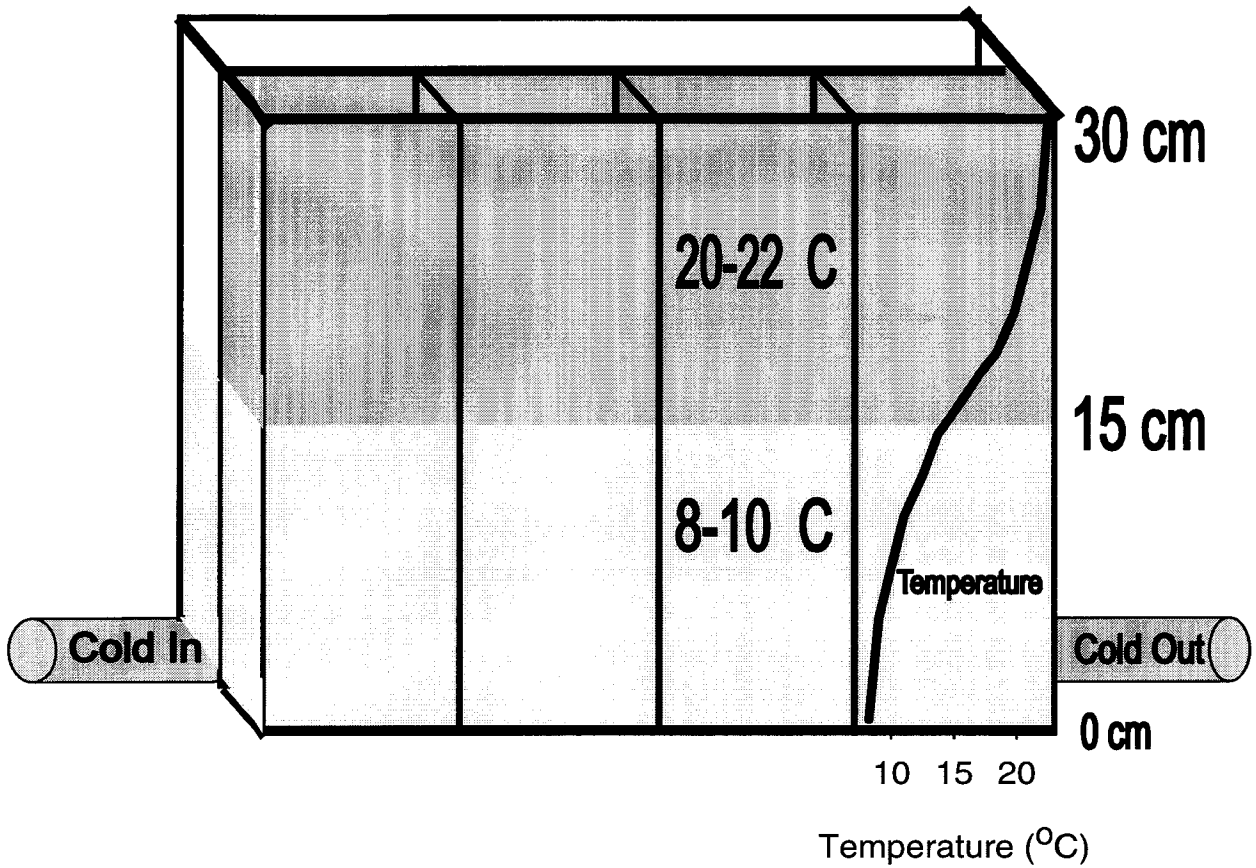

Figure 2. Schematic drawing of columns with flow-through cooling system. Cold water was pumped into the back section of the tank and returned to the tank by gravity. The cooled water from the tank is only allowed to fill half the tank in order to cool the bottom section only of the columns. The plot is a typical temperature profile for this system during experiments.

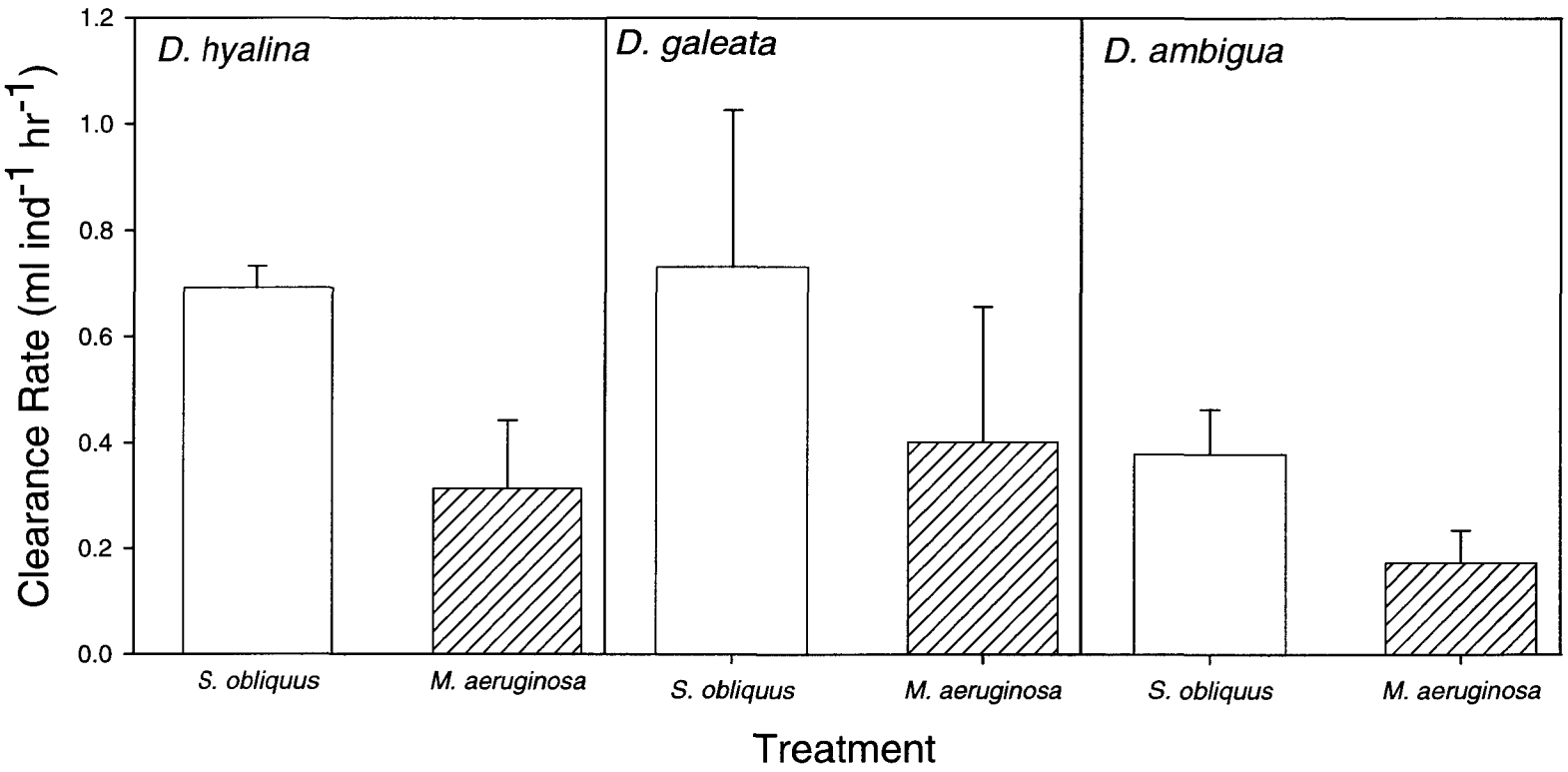

Figure 3. Clearance rates for all *Daphnia* fed either *Scenedesmus obliquus* or *Microcystis aeruginosa* over 3 h. The clearance rate for all *Daphnia* is approximately twice as high when fed *S. obliquus* than *M. aeruginosa.* Clearance rates were corrected to a body length of 1.5 mm for both *D. hyalina* and *D. galeata* and 1.0 mm for *D. ambigua.* The clearance rate for *D. hyalina* feeding on *S. obliquus* was significantly larger *M. aeruginosa* (p= 0.05, student t-test). For differences between the clearance rates for the other two species was not statistically significant (p=0.44 for *D. galeata*, p=0.12 *D. ambigua,* student t-test).

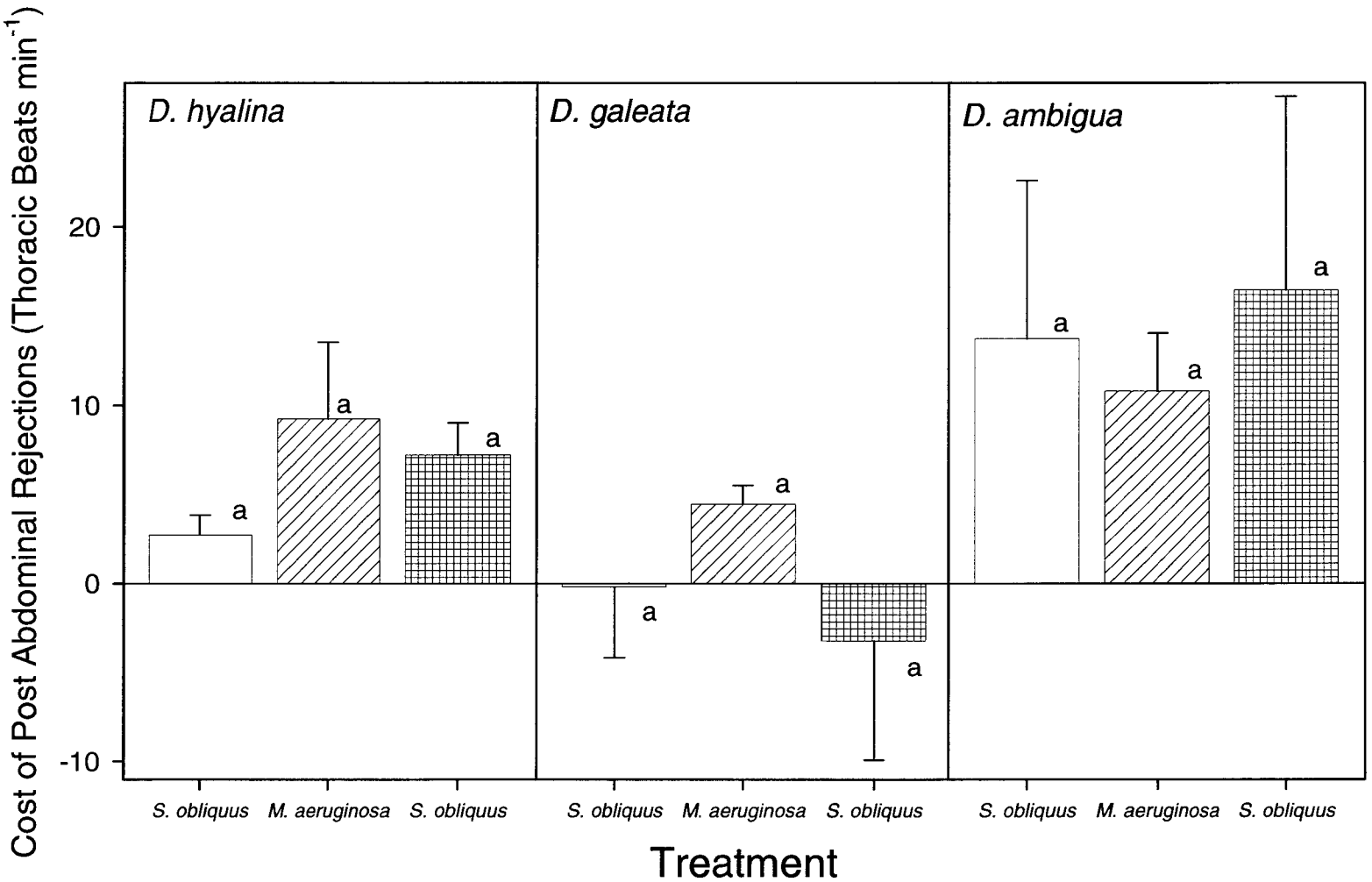

Figure 4. Cost on the rate of thoracic beats min^{-1} of post-abdominal rejections for three *Daphnia* species during a bioassay with *Scenedesmus obliquus* and *Microcystis aeruginosa.* The value of costs between treatments for each species were not statistically significant ($p>0.05$, Repeated measures ANOVA)

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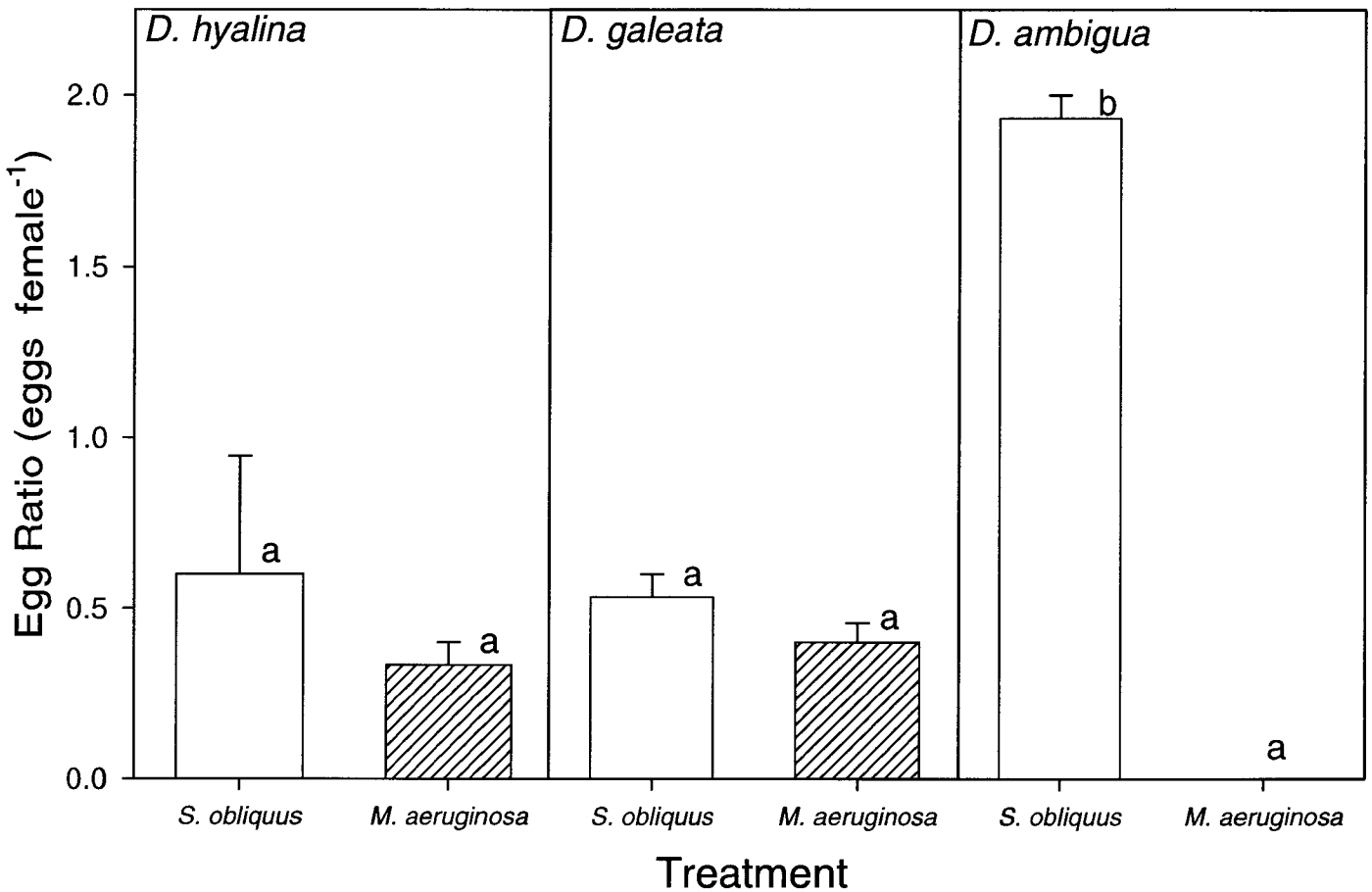

Figure 5. Eggs female^{-1} after 5 d of being fed either *Scenedesmus obliquus* or *Microcystis aeruginosa*. For each experiment, n=3 beakers of 3 to 5 animals each. Statistically, *D. ambigua* had significantly more eggs when fed *S. obliquus* than when *M. aeruginosa* (p=0.001, student t-test). For the other two species, there was no statistically significant difference between treatments.

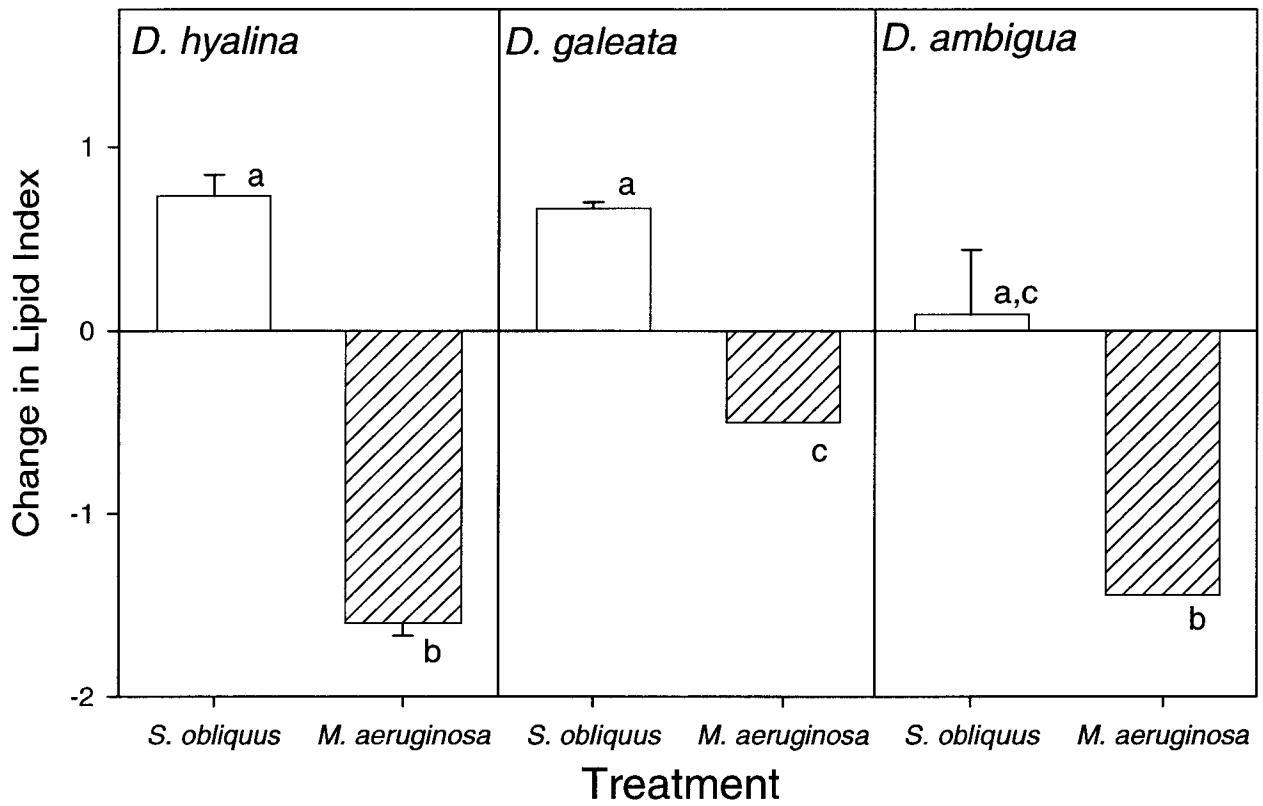

Figure 6. The change in the lipid index of three *Daphnia* species after 5 days of being fed either *Scenedesmus obliquus* or *Microcystis aeruginosa.* The amount of lipids increased in all daphnids fed *S. obliquus* and decreased in all daphnids fed *M. aeruginosa*. The differences were statistically significant within species. Similar letters indicate no significant difference between values. *D. galeata* had a significantly smaller change in lipids than either *D. hyalina* (p= 0.003) or *D. ambigua* (p=0.010) when all three were fed *M. aeruginosa* (ANOVA, Tukey post-hoc test).

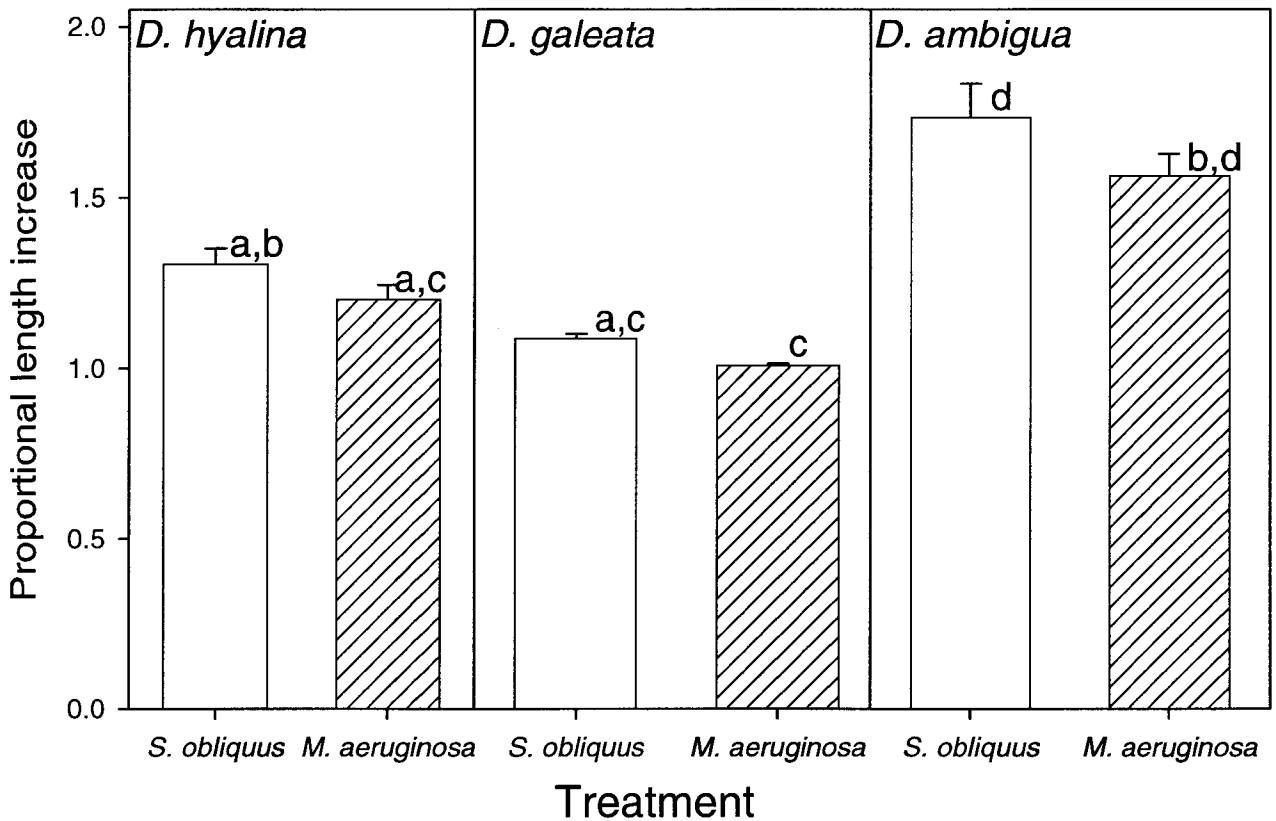

Figure 7. The mean proportional increase in length of three *Daphnia* after 5 days of feeding on either *Scenedesmus obliquus* or *Microcystis aeruginosa* at 1 mg C L^{-1}. For each experiment, n=3 beakers of 3 to 5 animals each. Same letters indicate no statistically significant difference between values. No statistically significant difference was seen between treatments within species; however, the data suggest a greater proportional length increase in animals fed *S. obliquus* than animals fed *M. aeruginosa*.

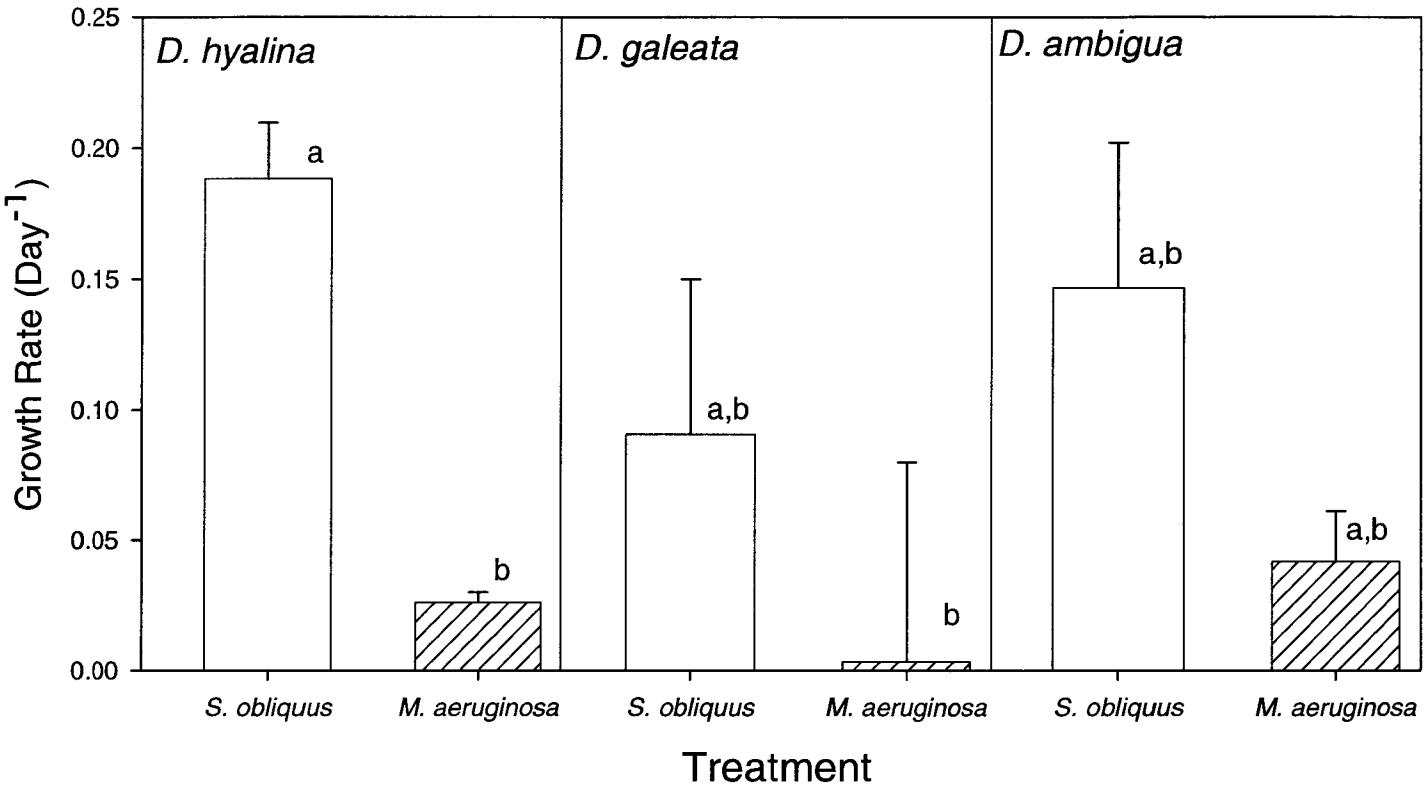

Figure 8. Daily growth rate of *Daphnia* after 5 days of being fed either *Scenedesmus obliquus* or *Microcystis aeruginosa*. For each experiment, n=3 beakers of 3 to 5 animals each. *D. hyalina* fed *S. obliquus* grew significantly faster than when fed *M. aeruginosa* (p=0.002, ANOVA, Tukey post hoc test). Same letters indicate no significant difference between values.

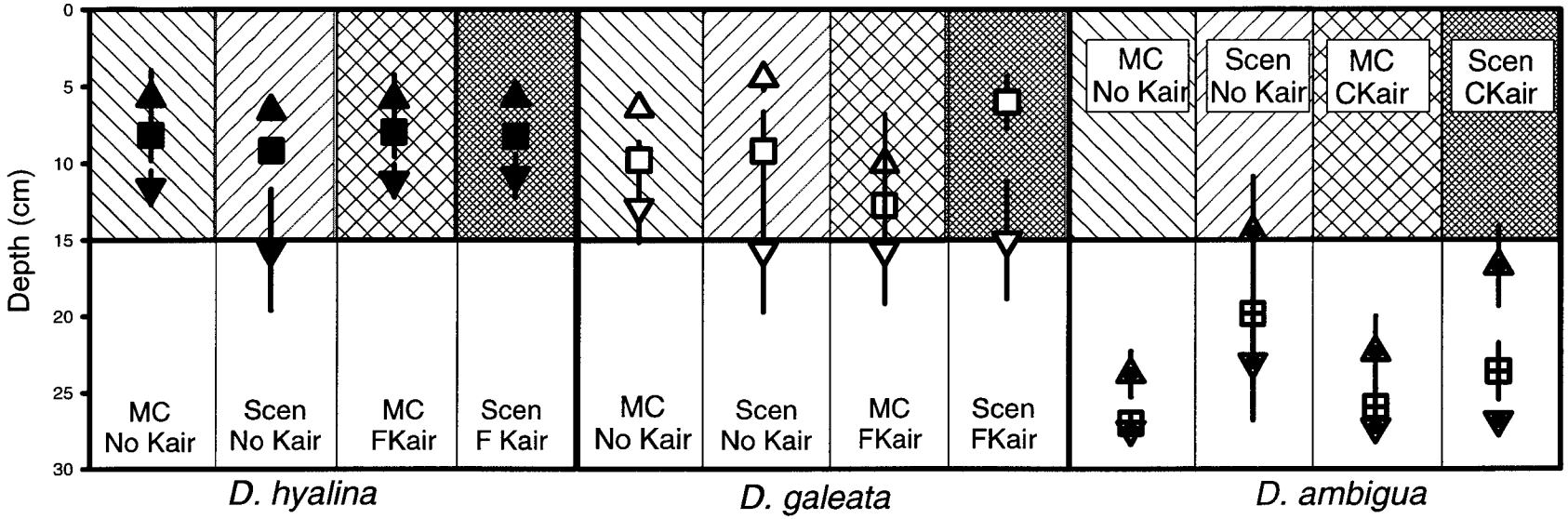

Figure 9. Quartile depth distributions of three *Daphnia* species over 6 h when in 30 cm columns filled with water of four conditions. Water used was either filtered well water (NoKair) or filtered water infused with kairomones from either fish (FKair) or *Chaoborus punctipennis* (CKair). The 25% quartile is represented by up-pointing triangles (▲). The 50% quartile is represented by the square (■). The 75% quartile is represented by down-pointing triangles (▼). Each column was given either 1 mg C L^{-1} of *Scenedesmus obliquus* (Scen) or 0.5 mg C L^{-1} of both *S. obliquus* and *Microcystis aeruginosa* (MC). The middle horizontal line indicates the thermocline which was kept at 15 cm throughout the experiment. The vertical lines connected to the symbols indicate one standard error.

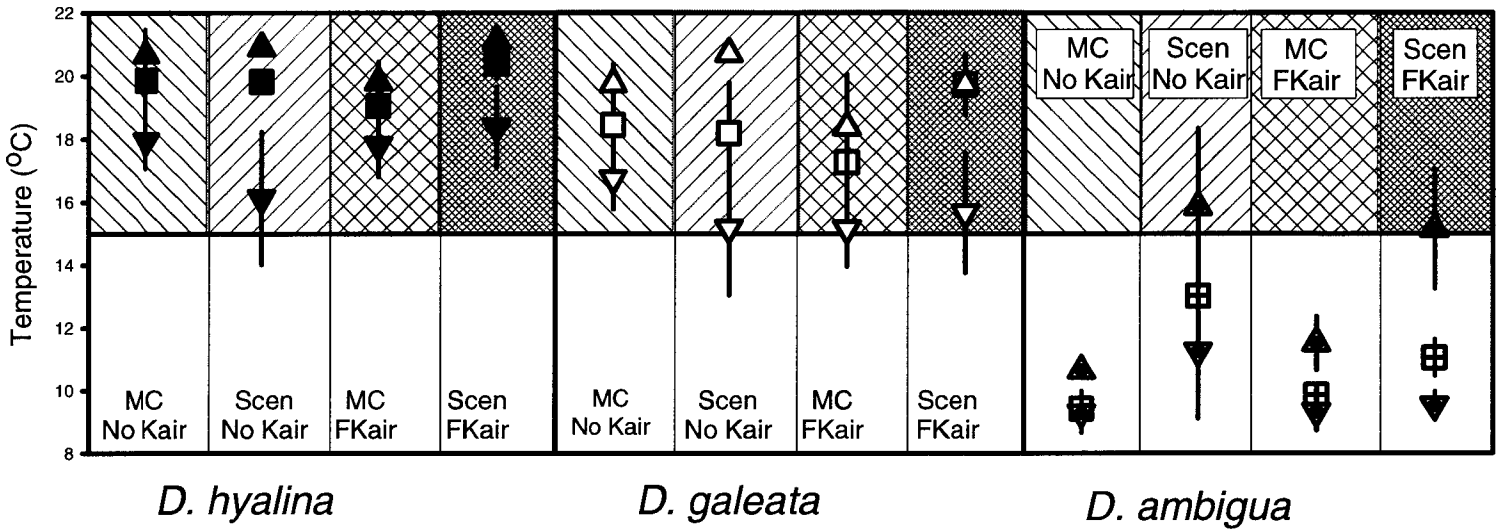

Figure 10. Temperature quartiles of three *Daphnia* species over 6 h when in 30 cm columns in of the four treatment conditions. Water used was either filtered well water (NoKair) or filtered water infused with kairomones from either fish (FKair) or *Chaoborus punctipennis* (CKair). The 25% quartile is represented by up-pointing triangles (▲). The 50% quartile is represented by the square (■). The 75% quartile is represented by down-pointing triangles (▼). Each column was given either 1 mg C L^{-1} of *Scenedesmus obliquus* (Scen) or 0.5 mg C L^{-1} of both *S. obliquus* and *Microcystis aeruginosa* (MC). The middle horizontal line indicates the thermocline (15 cm, approximately 15° C) throughout the experiment. The vertical lines connected to the symbols indicate one standard error. For all three species, the widest range of temperatures experienced was by the animals fed *S. obliquus* and in water without predator kairomones.

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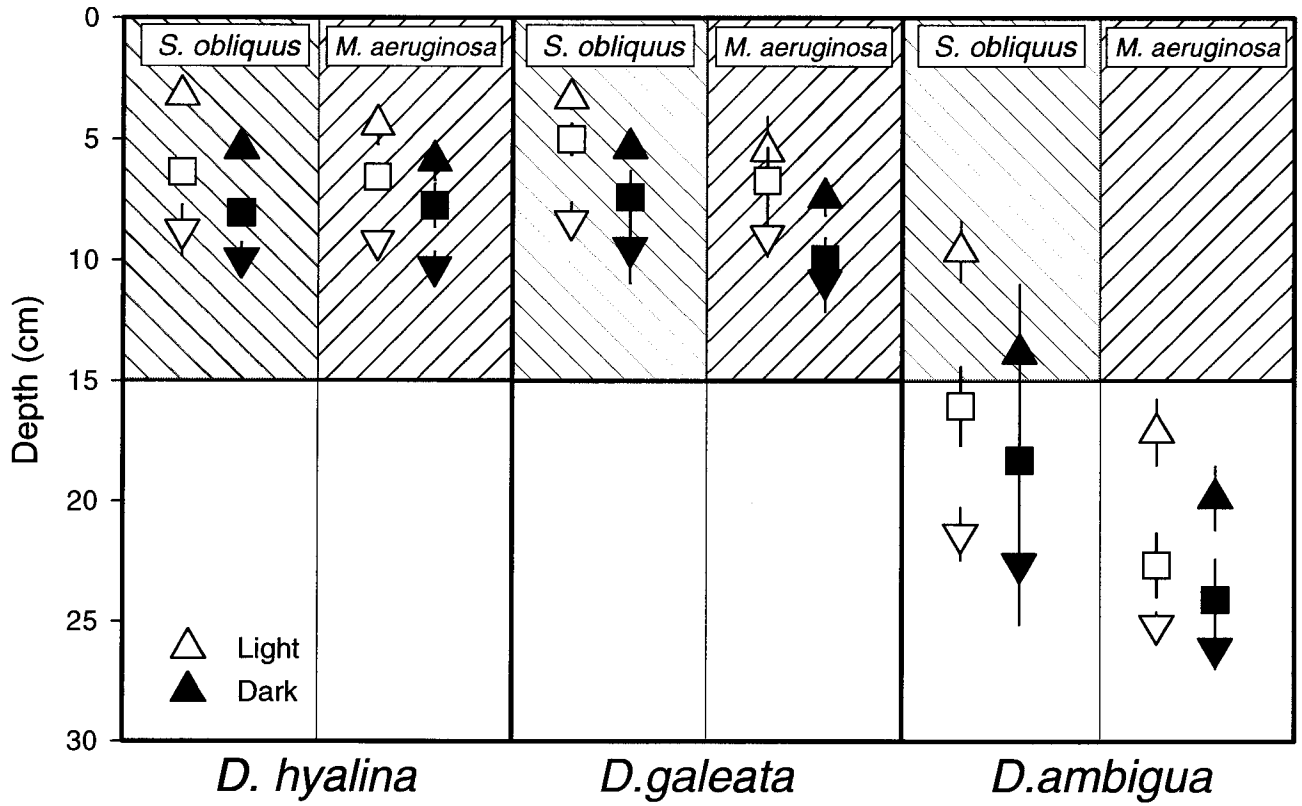

Figure 11. Quartile depth distributions of three *Daphnia* species over 3 d with simulated sunrises and sunsets. Each column was filled with kairmone infused filtered water. For *D. hyalina* and *D. galeata*, the kairomone used was from *Lepomis gibbosus* (pumpkinseed sunfish. For *D. ambigua,* the kairomone was from *Chaoborus punctipennis* (phantom midge larvae). Each column either 1 mg C L^{-1} of *Scenedesmus obliquus* (*S. obliquus*) or 0.5 mg C L^{-1} of both *S. obliquus* and *Microcystis aeruginosa* (*M. aeruginosa*) added to the epilimnion as a food source. The middle horizontal line indicates the thermocline which remained at 15cm ±1 cm throughout the experiment. The 25% quartile is represented by up-pointing triangles (▲). The 50% quartile is represented by the square (■). The 75% quartile is represented by down-pointing triangles (▼). The vertical lines extending from the symbols indicate one standard error.

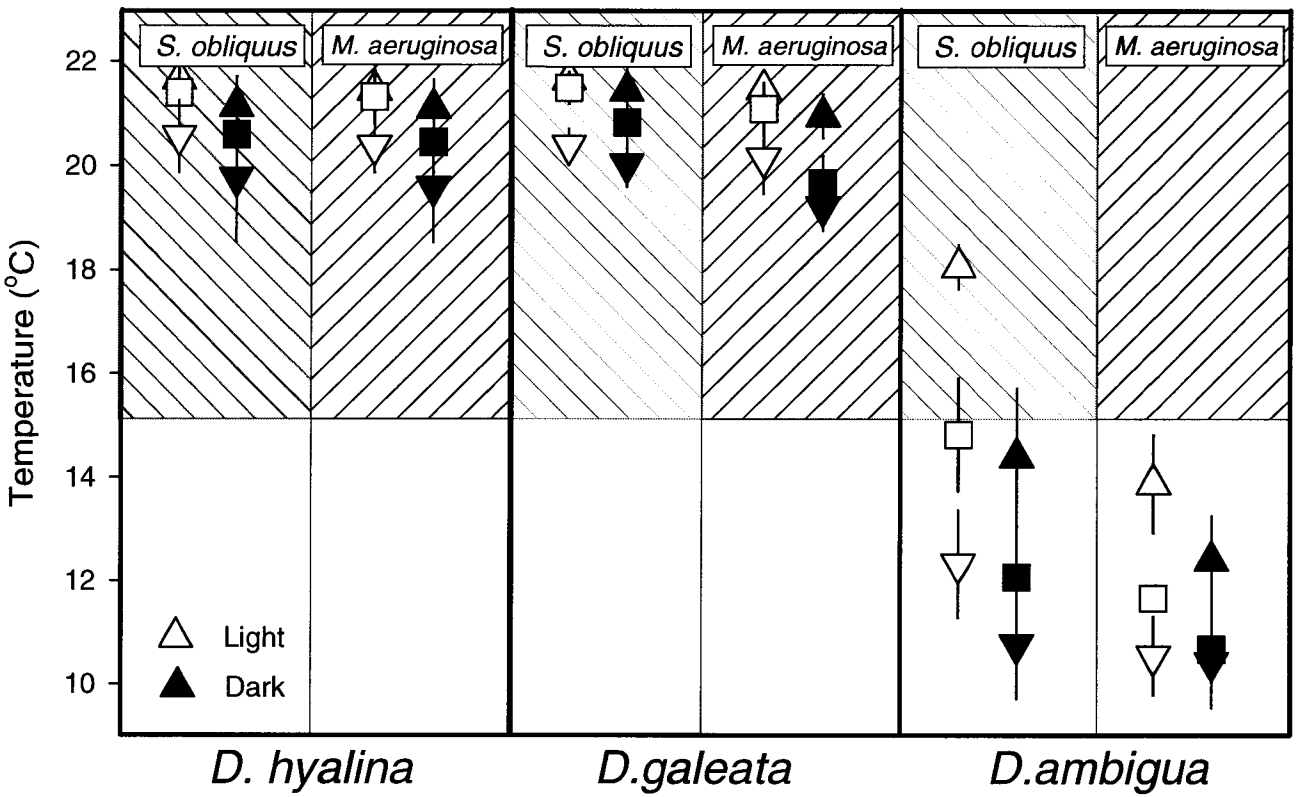

Figure 12. Quartile temperature distributions of three *Daphnia* species over three days with simulated sunrises and sunsets. For *D. hyalina* and *D. galeata*, the kairomone used was from *Lepomis gibbosus* (pumpkinseed sunfish. For *D. ambigua,* the kairomone was from *Chaoborus punctipennis* (phanotom midge larvae). Each column had either 1 mg C L^{-1} of *Scenedesmus obliquus* (*S. obliquus*) or 0.5 mg C L^{-1} of both *S. obliquus* and *Microcystis aeruginosa* (*M. aeruginosa*) added to the epilimnion as a food source. The middle horizontal line the thermocline which remained at 15 °C ±1 °C throughout the experiment. The 25% quartile is represented by up-pointing triangles (▲). The 50% quartile is represented by the square (■). The 75% quartile is represented by down-pointing triangles (▼). The vertical lines extending from the symbols indicate one standard error.

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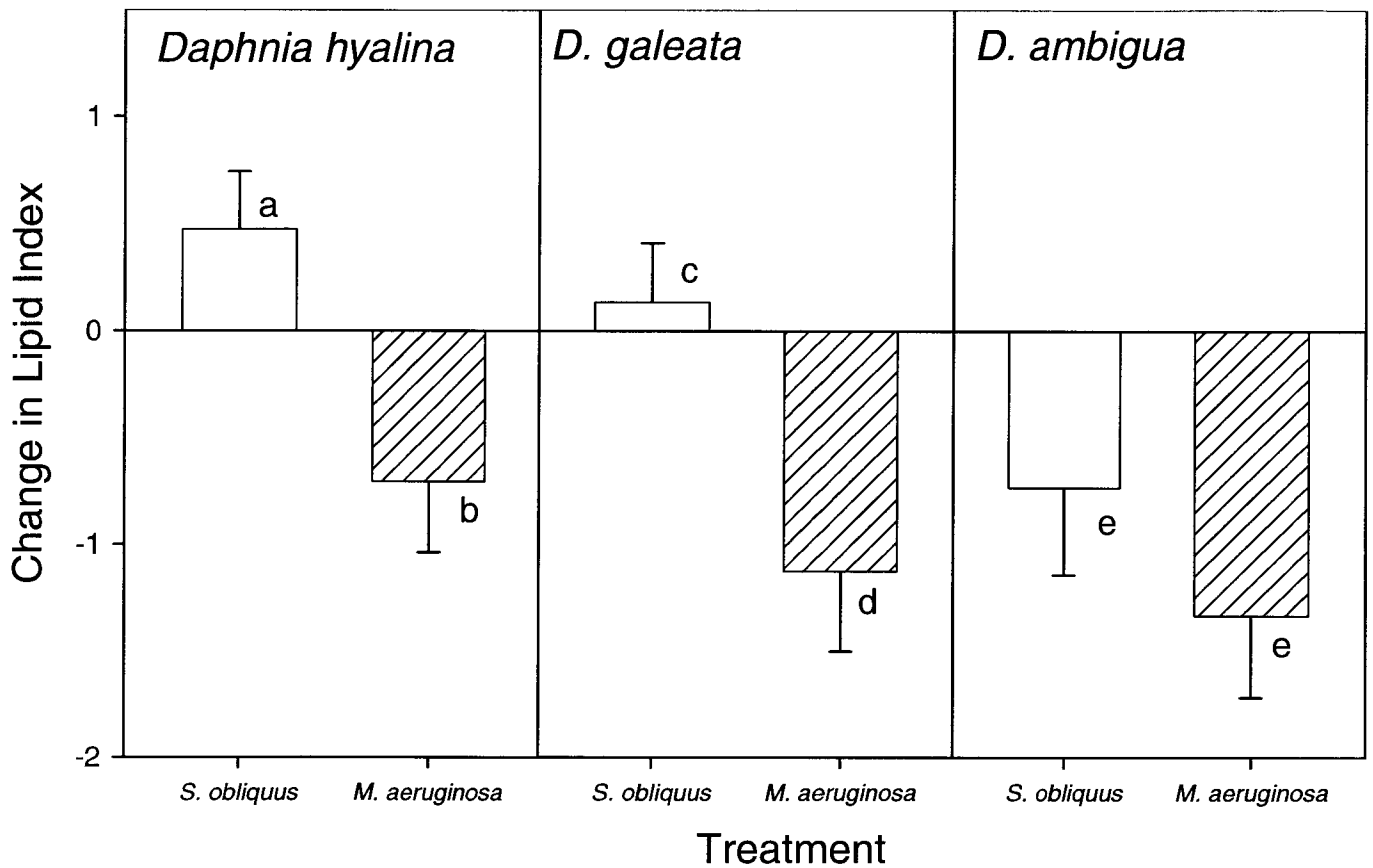

Figure 13. The change in the lipid index of *Daphnia* after 3 d in a column with either 1 mg C L-1 of *Scenedesmus obliquus* or 0.5 mg C L-1 of both *S. obliquus* and *Microcystis aeruginosa.* For each experiment, n=3 replicates of 10 animals in each. All daphnids had an increase in lipids when fed *S. obliquus* and a decrease in lipids when fed *M. aeruginosa*. *D. hyalina* and *D. galeata* experienced an increase in lipids when fed *S. obliquus* and a loss of lipids when fed a mixture of food; the differences were statistically significant within species (p=0.05 for both species). Student t-test). *D. ambigua* had a reduction in lipids in both treatments (p=0.35, student t-test). Similar letters indicate no significant difference between values.

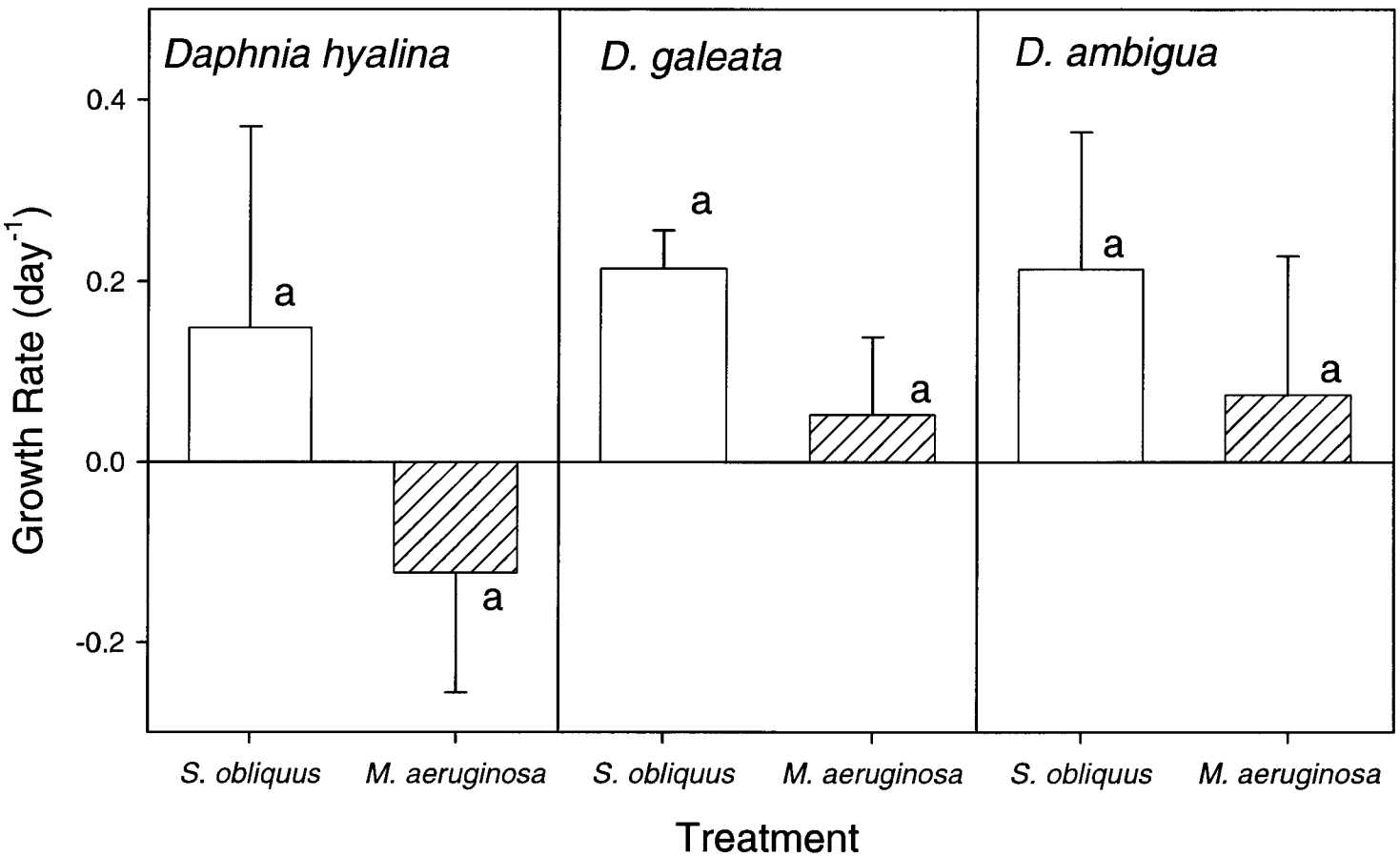

Figure 14. Growth rate of *Daphnia* after 3 days in a column with either 1 mg C L^{-1} of *Scenedesmus obliquus* or 0.5 mg C L^{-1} of both *S. obliquus* and *Microcystis aeruginosa.* Although not statistically significant, all *Daphnia* grew faster when fed *S. obliquus*. Similar letters indicate no significant difference between values.

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APPENDIX

Light Control Program for Basic

```
1 REM RUNLIGHTS.bas
10 DEFINT A
20 INPUT "Enter number file name: ",N$
30 OPEN N$ FOR INPUT AS #1
40 INPUT #1, N
45 print "Number of Values: ", N
50 DIM VALUE(N)
60 PRINT, "Reading values..."
61 rem values should be integers between 0 and 1023
65 FOR X = 1 TO N
70 INPUT #1, VALUE(X-1)
80 NEXT X
90 CLOSE #1
110 INPUT "Enter time between entries (in seconds): ",T
140 FOR X = 1 TO N
149 rem must multiply input value by approx 4 to get dimmer value 0 to 4095
150 A = 4*VALUE(X-1) : GOSUB 16000
160 rem Wait for specified time
170 for W = 1 to T
171 rem S range is set to approximate 1 second.
172 rem NOTE S varies if ran on different computer
173 for S = 1 to 7700
174 next S
180 next W
195 PRINT "TIME:", X, "VALUE=", value(x-1)
210 NEXT X
230 END
16000 OUT 768, PEEK(VARPTR(A))
16010 OUT 769, PEEK(VARPTR(A)+1)
16020 OUT 770, PEEK(VARPTR(A))
16030 OUT 771, PEEK(VARPTR(A)+1)
16040 OUT 772, PEEK(VARPTR(A))
16050 OUT 773, PEEK(VARPTR(A)+1)
16060 OUT 774, PEEK(VARPTR(A))
16070 OUT 775, PEEK(VARPTR(A)+1)
16080 OUT 776, 0
16090 RETURN
```